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SENSITIVITY OF THE RHESUS MONKEY CORNEA AND SURROUNDING TISSUES TO HEAT PRODUCED BY CO_2 LASER RADIATION

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INTRODUCTION

The increased use of carbon dioxide (CO_2) laser systems by the military has led to studies (1,2,3) which identified the minimum amount of energy necessary to produce skin or corneal alterations. The data resulted in the establishment of 100 milliwatts/cm^2 (mw/cm^2) as the maximum permissible exposure (MPE) level for humans to this 10.6 micron radiation. This figure represents the "safe" irradiance for direct viewing by humans for times in excess of ten seconds. The research provided the median corneal damage threshold for rabbits, owl monkeys, and rhesus monkeys. The safe exposure levels were then extrapolated and published in TB Med 279 (4).

Recent research (5), has shown that when animals are exposed over varying periods of time to "safe" levels of radiation from lasers emitting energy in the visible portion of the electromagnetic spectrum, several behavioral changes can be observed, particularly in those responses dependent upon color vision. One interpretation of these results has been that the behavioral methods of evaluating visual or other functional changes are more sensitive indicators of functional changes than the techniques formerly used to establish the "safe" levels of laser exposures.

Since CO_2 laser radiation is in the far infrared and is therefore invisible, its presence is signaled only by the sensation of heat at levels below those shown to produce visible tissue change. In laboratory and in field situations, exposures of individuals to this low level irradiance is a distinct possibility. The purposes

of the present study were to determine the irradiance level at which the rhesus monkey could detect 10.6 micron radiation at the cornea, lids and surrounding facial tissue, to establish the differential sensitivity of these tissues to thermal stimulation and to examine the effects of repeated low level radiation doses upon the performance of the rhesus monkey.

The absorption of CO_2 radiation by both the cornea and skin is essentially the same (6). In both cases over 95% of the initial radiation is absorbed in the first 50 microns of the tissue. One of the major differences between these two structures is to be found in their sensory receptor systems. The skin contains for the most part encapsulated end organs such as Ruffini cylinders and Krause end bulbs which have been shown to subserve the sense modalities of warmth and cold, respectively. The stromal layer of the cornea, by contrast, was found by Zander and Weddell (7) to contain only free nerve endings with beaded axons. The corneal epithelium was found to contain many more axons than those in the stroma. These were described as being very fine, unspecialized nerve endings which lay between the epithelial cells.

Classical studies of thermal sensation in the human cornea have led to disagreement on the sensation of heat in this structure. Earlier researchers, such as Von Frey (8) concluded that the only sensation subserved by the cornea was that of pain. Lele and Weddell (9) reported that brass cylinders whose temperature was 1.5° C above or below the corneal temperature, as well as near infrared radiation focused on the cornea, gave rise to reports of warmth or cold. These authors (10) further studied the sensitivity of the fine corneal axons by recording electrophysiological activity following irradiance of the cornea with a near infrared source. They noted an increase in the discharge activity of the neural preparation as the strength of the stimulus was increased. Subsequently, Kenshalo (11) reported that chilled and heated cylindrical stimuli applied to the cornea evoked reports of greater or lesser degrees of irritation, not heat or cold. Kenshalo's data leads to the inference that free nerve endings are sensitive to changes in thermal stimuli, but that the sensations evoked were not perceived by the subjects as temperature variables.

In the present study, rhesus monkeys, whose corneas, lids and skin are analagous to those of humans, were used in an attempt to determine the responsivity of these tissues to heat produced by the CO_2 system. The term "heat" in the present context refers only to temperature rise in the tissues exposed and not to the percept of the rhesus monkey.

Few studies have been reported in which the threshold sensitivity of the rhesus monkey to thermal stimuli has been determined. Of these, the primary emphasis has been on responses to temperature increase or decrease and differential heat and cold thresholds. Berkeley and Hughes (12) discussed the inordinate difficulties and failures they encountered in attempts to train monkeys to discriminate between hot and cold stimuli. Smith (13), however, reported the use of a conditioned suppression technique to determine the sensitivity of the rhesus to increases and decreases in temperature. No thresholds were reported in this study. Laursen (14) trained three green monkeys (Cercopithecus Aethiops) to report their differential thresholds for temperature sensations while grasping a heated or cooled cylinder. His findings indicated that at 20° C, the differential thresholds were the same (approximately 1° C) for humans and monkeys. The differences increased to about 6° C more for monkeys at the upper limits (39° C) of the temperatures used.

The use of the CO_2 laser to produce heat stimuli offers several distinct advantages. Both the irradiance and exposure duration can be accurately controlled and varied to provide complete flexibility. Corneal irradiance diameters can be chosen to meet any experimental or theoretical consideration. Since, as was previously indicated, over 95% of the invisible 10.6 micron irradiation is absorbed in the first 50 μ of corneal or other tissue, the heat stimulus can be introduced to the cornea with no experimental artifact. Earlier corneal sensitivity results were confounded by mechanical contact of the cornea with hairs and cylinders or by the use of broad-banded IR sources where the absorption occurred in deeper lying tissues and structures, such as the corneal stroma, anterior chamber, and iris.

METHODS

Subjects

Two rhesus monkeys were trained using the procedures described in this report. They were males, approximately 3½ years of age. No abnormalities of the cornea or adjacent tissue were noted in these animals.

Behavioral Training

The training of each animal was divided into three phases. In the first, the animal was chronically chaired in a primate cubicle (BRS/LVE 132-12). He was then taught to feed himself, and by

the method of successive approximations learned to depress a lever placed to his right for a drop of orange juice substitute. Within a day the animals bar pressed for reinforcement at a variable ratio of 2 (VR2). The ratio was gradually reduced until the animal bar pressed at a constant rate of speed on a VR10 schedule. This required approximately one week during which the animal's training was divided into three one-half hour sessions per day. Two additional weeks were then used to accustom the animal to an eyepiece and plexiglass head restraint system which was fitted to his particular head shape. During this time, baseline bar pressing rates were obtained under free-run (no head restraint, heat or shock) and "sham" (no heat or shock) conditions.

The second phase of the experiment consisted of exposing the animal for 20 seconds to a 200 mw/cm^2 irradiance through an 8 mm aperture. Termination of the beam by a shutter coincided with a 2.0 milliampere shock applied between a bracelet attached to the animal's leg and a chain secured across the animal's waist. In a single one-half hour session the animal ceased responding as soon as radiation from the laser source was detected. This phase of response suppression training lasted approximately 14 days, and consisted of two one-half hour sessions devoted to exposures at the 200 mw/cm^2 condition and a third sham session per day.

The third phase of the training paradigm required the monkey to respond to 100, 50, 25, 10, and 1 mw/cm^2 stimuli with 4, 8, and 16 mm beam diameters. In this training sequence, however, no shocks were given at 10 or 1 mw/cm^2, and 100% of the time at the higher irradiances.

Circuit Description

Figure 1 is a simplified block diagram of the training system. A 30-minute timer was activated to start each session. The animal pressed a non-retractable lever placed to his right in the enclosed cubicle. Two separate circuits were used for each cubicle. In the first, bar pressing activated a random probability generator set at 10%, which in turn activated a liquid solenoid yielding positive reinforcements of approximately .01 ml per pulse.

The second system, also activated by the lever, was connected to a reset counter set for the average number of bar presses the animal normally made in a 20-second period of time. This was based upon his bar pressing rate in phase two of the training and varied for each animal. When the reset counter was activated by the prescribed number of bar presses, a second probability generator,

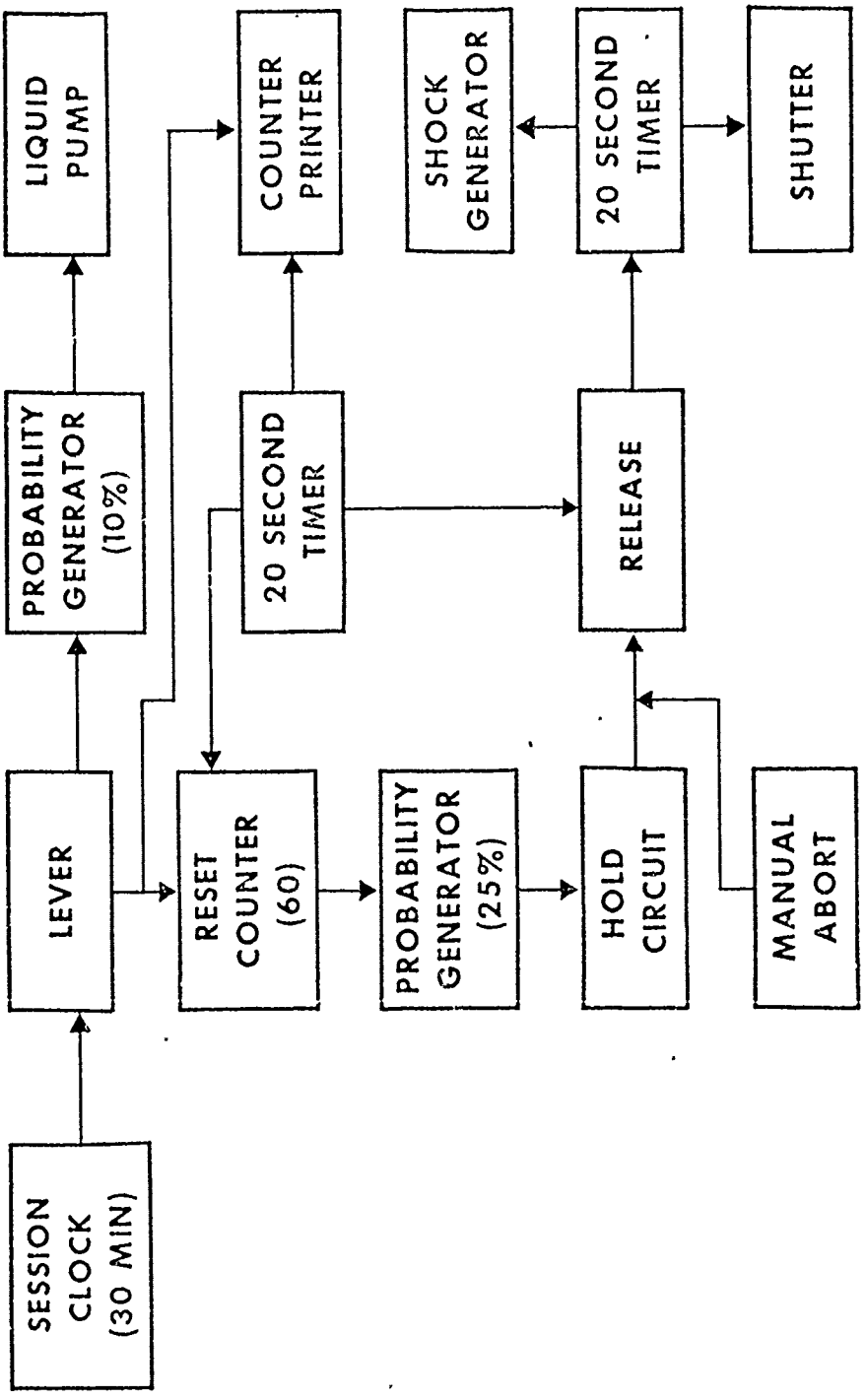

Figure 1. Block diagram of conditioned suppression behavioral training and testing system.

operated at 25%, was triggered. This in turn armed a "hold" circuit. A 20-second timer then simultaneously triggered a "release" circuit and a counter-printer (Grason-Stadler Model E12405A), which printed out the number of bar presses in the preceding 20-second period of time and reset the counter to zero. The release circuit activated a second 20-second timer, which in turn opened an electronic shutter, allowing the CO_2 radiation to pass into the cubicle. At the conclusion of this 20-second interval, an end-pulse triggered the shock generator.

The counter-printer recorded not only the number of bar presses in each 20-second interval, but also each shutter opening and each shock. A multipen event recorder was also used to directly visualize the advent of each 20-second period, the pattern of bar presses in this period, the onset of the CO_2 laser, and the liquid reinforcements.

While the above system was automatic, it was occasionally necessary to control exposures by hand, especially during the 1, 10, and 25 mw/cm^2 conditions. This was done by way of an "abort" system which operated a second electronic shutter that blocked the CO_2 radiation and simultaneously turned off the shock generator. Sham trials were thus easily introduced during each session. Baseline data was run on days six and seven of each week.

Each animal's behavior was observed during the trials by way of a closed circuit television system (CCTV).

Laser System

A linearly polarized 3 watt CO_2 laser (Sylvania, Model 941S) operating in the TEM_{oo} mode at 10.6 microns was used as the stimulus source. A block diagram of the optical system is presented in Figure 2. A variable attenuator (Sylvania, Model 485) coupled to the output of the laser was used to adjust the power to select the desired irradiances. A gold leaf safety shutter reflected the beam into detector I (Coherent Radiation Labs, Model 201) whose output was monitored by the dual-pen chart recorder (Honeywell, Electronik 194). This system allowed safe termination of the beam between experimental sessions and provided a continuous record of the stability of the beam amplitude. Two gold-coated electronic shutters (Uniblitz, Model 225X2A2) controlled the delivery of the laser energy. A 4.5 cm germanium beam splitter with a reflectivity of 35% and transmissivity of 62% allowed accurate monitoring of a portion of the exposure power. The radiant power from the reflected portion of

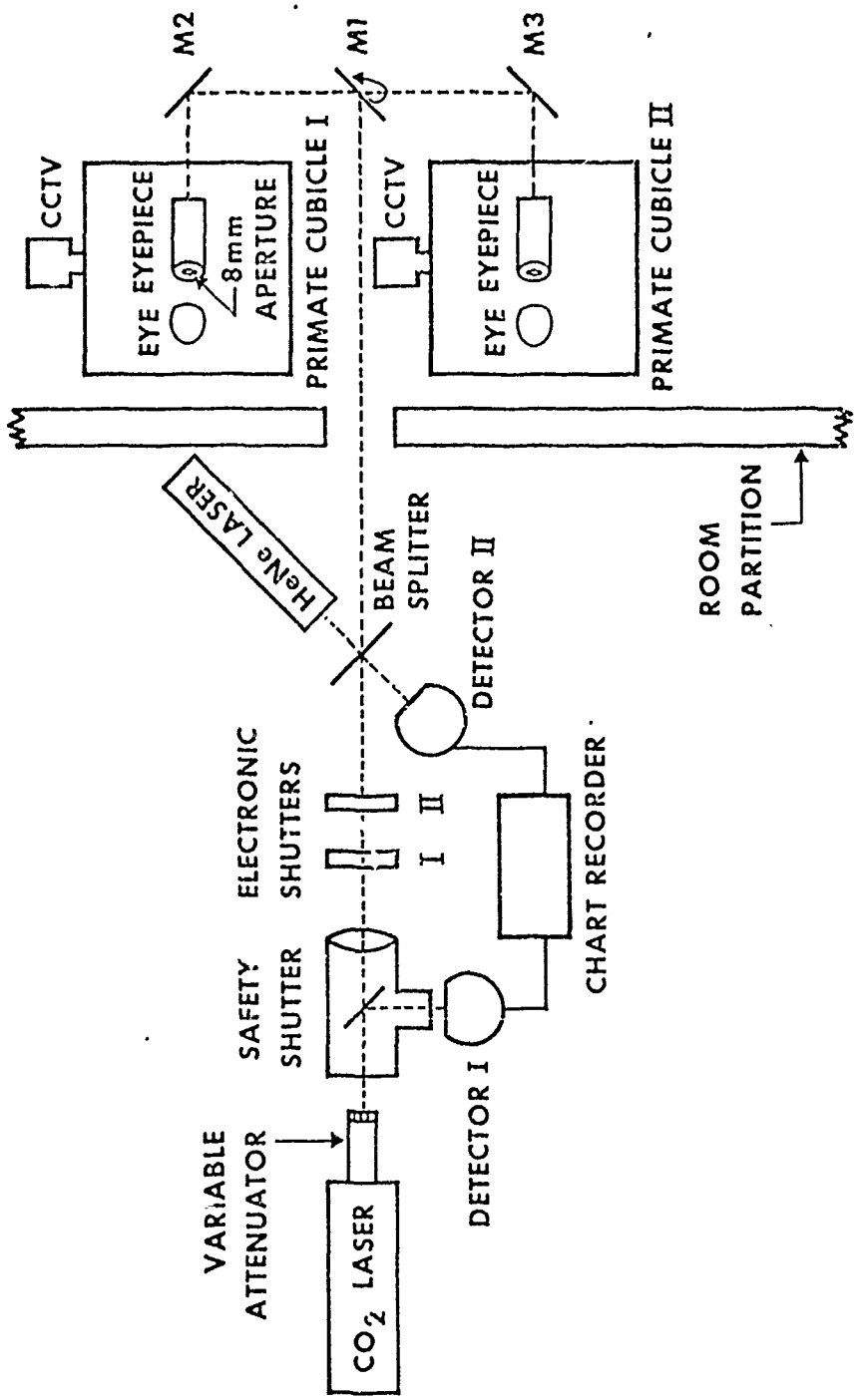

Figure 2. Schematic of laser and optical system.

the beam was measured by detector II (CRL, Model 201) and also recorded on the dual-pen chart recorder. A 0.5 mw helium-neon (HeNe) laser was aligned co-linearly with the CO_2 laser beam by using the reflection from the back surface of the beam splitter. The HeNe laser was turned off during the test sessions. The laser system, optics, and electromechanical programming systems were placed in a room adjacent to the animal cubicles. This eliminated auditory cues and provided precise external stimulus control. The germanium beam splitter was opaque in the visible region of the spectrum and completely masked the 2.5 cm shutter opening, thereby eliminating visual cues. The delivery system and cubicles were arranged such that two animals could be tested in sequential sessions with the rotation of only one mirror (M1, Figure 2). Flat front surface mirrors in precision mounts were attached to platforms on the cubicle doors and reflected the beam down a 15 cm tube aligned with the subject's eye. The interchangeable apertures were located 3 cm in front of the subject's eye and 486 cm from the front of the CO_2 laser. The inherent divergence of the CO_2 laser (4.5 mrad) was used to expand the beam. The intensity distribution of the beam at the aperture plane was Gaussian with a diameter of 16 mm at the 1/e intensity points.

Alignment and Calibrations

Each week the animals were removed from their cubicles so that irradiance measurements could be made at the eye position. An eight-junction bismuth-silver thermopile (Eppley Laboratories) and power monitor (CRL, Model 201) whose calibration was traceable to the NBS were used. A calibration curve of the irradiance at the eye as a function of the chart recorder reading from detector II was obtained by systematically adjusting the attenuator. Each week's calibration curve was within 5% of the previous week's curve. Reproducibility of the irradiance required careful alignment of the CO_2 laser beam, thus prior to each exposure session the co-linearity of the HeNe and CO_2 laser beams was checked and adjusted at two points separated by at least two meters. The irradiance was then selected by adjusting the attenuator to the predetermined reading on the calibration curve. The intensity distribution at the eye behind the 8 and 4 mm apertures was nearly uniform (within 5-8%) with some enhancement due to near field diffraction effects from the apertures used. No aperture was used for the 16 mm beam diameter condition.

RESULTS AND DISCUSSION

A sample of the data recorded during several trials for the 8 mm diameter aperture condition is presented in Figure 3. Part A

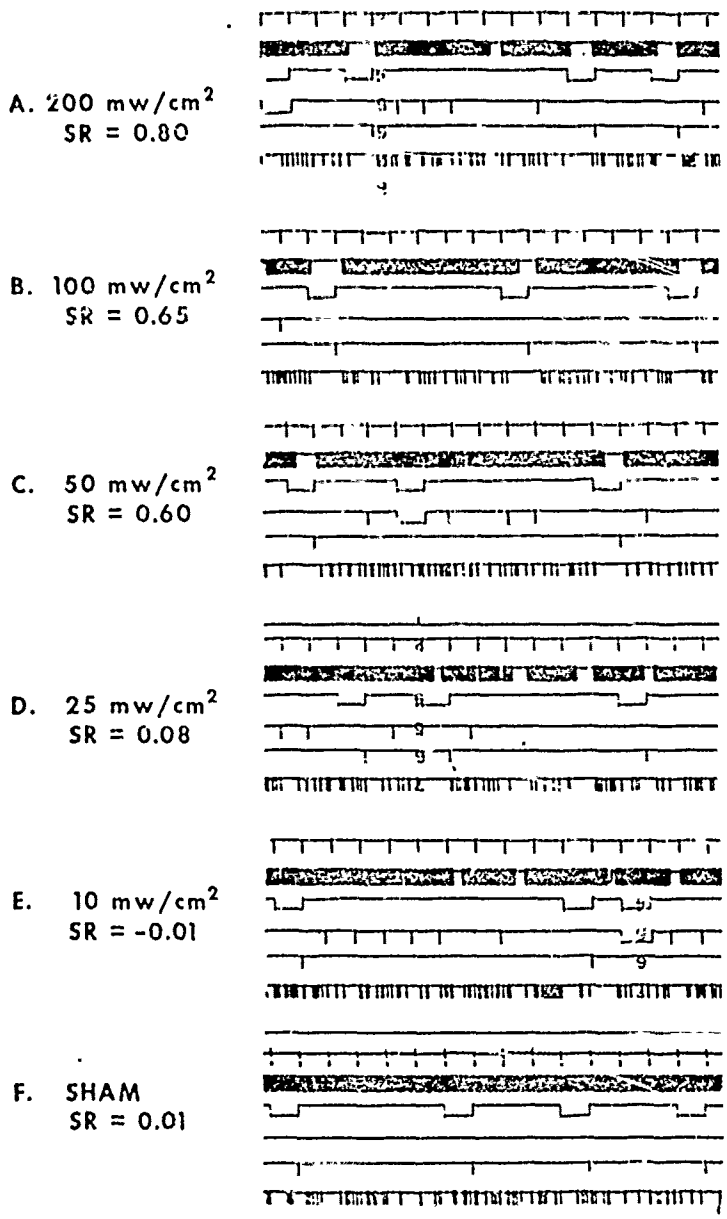

Figure 3, Sample of strip chart records of the suppression of bar pressing behavior in the presence of CO_2 irradiation. The suppression ratios for each irradiance level in this sample are presented.

is an example of the data at the 200 mw/cm^2 CO_2 irradiance level. The intervals between pen deflections in the top line represent 20-second time periods. The second line shows the actual bar presses which the animal made during each 20-second interval. Shutter opening to initiate a 20-second exposure is shown in the next line. The following line represents the abort system, in which the second shutter was closed, thereby blocking the CO_2 radiation. This resulted in sham trials during each run (Part A, C and E). The next lines show, respectively, the shock and the positive reinforcement events which occurred during the course of the trials.

From this data a measure of the animal's performance, the Suppression Ratio (SR), was obtained. This ratio was computed by subtracting from the number of bar presses in the 20-second interval immediately preceding the exposure (A), the number of bar presses during the exposure (B), and dividing this result by the number of bar presses occurring in the 20-second period prior to the exposure (A-B)/A. Thus, an SR approaching 1.00 represents complete response suppression, while an SR approaching zero denotes lack of sensitivity of the animal to the stimulus. In the present studies, the suppression ratio which represented CO_2 radiation sensitivity at the cornea and surrounding tissues would never be 1.00 (no bar pressing during the exposure), since thermal sensitivity depends upon the rate and area of absorption of the stimulus. In this experiment at the maximum irradiance level studied, several seconds were required for the animals to report the presence of the stimulus by the cessation of bar pressing.

Figure 4 represents the mean suppression ratios at each irradiance for each beam diameter used in the present study for one animal. Data from the second animal showed no differences. The horizontal line drawn at the .36 SR point represents two standard deviations above the mean for the sham exposures ($\overline{X}$ = .06). This point was selected based upon two criteria. First, the maximal suppression ratio depends upon the absorption of heat in the tissues and the subsequent response of the animal. It was found that the SR for the most intense irradiance for the skin with the 16 mm beam was .84, or in terms of reaction time, the animal required approximately 3.2 seconds to respond to the stimulus. If this point is the lower limit of the reaction time of the monkey's response, a correction factor is required to normalize the data in order to establish thresholds. This can be accomplished in one of two ways: (a) by setting the maximum SR equal to 100% and plotting the other points as relative SR's; (b) by setting the baseline lower by the difference between 100% suppression and the maximum obtainable SR. In this case, this level coincided with the two standard deviation unit point and

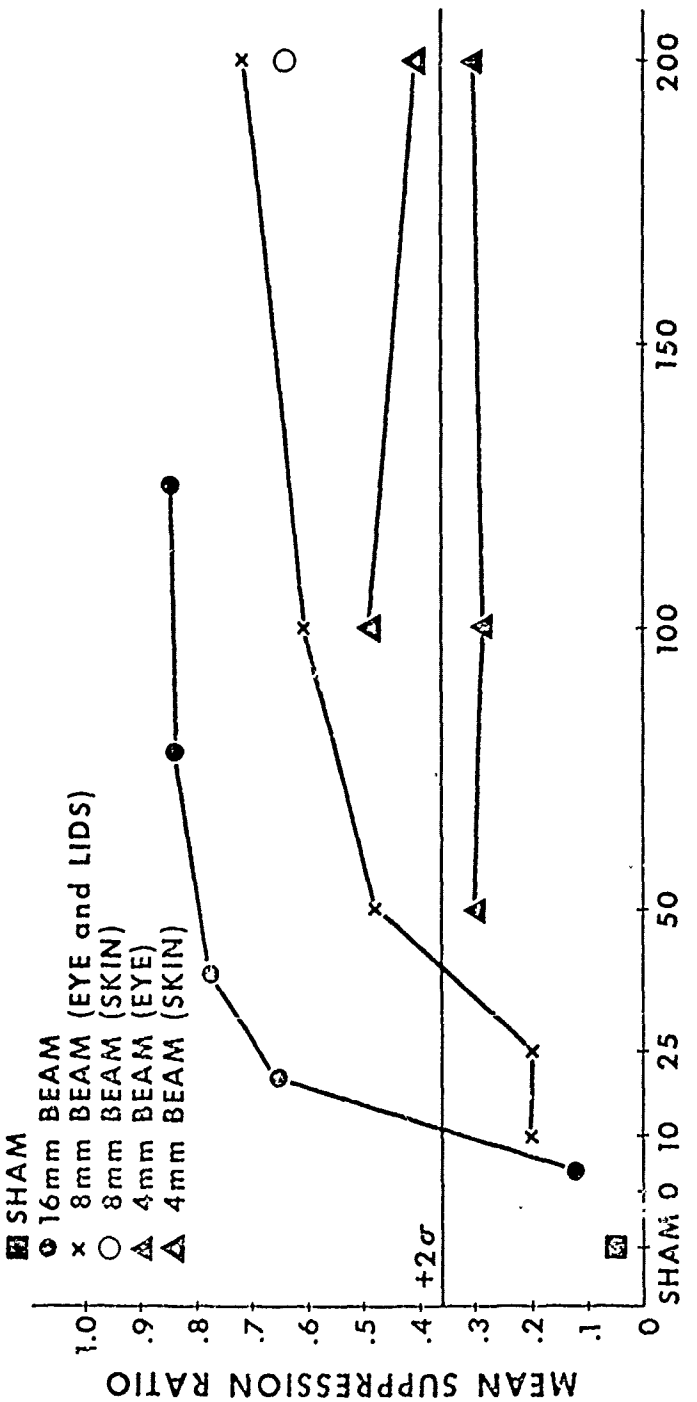

Figure 4. Mean Suppression Ratio at each irradiance for 4, 8 and 16 mm CO_2 radiation beam diameters.

was thus considered the more reliable and accurate representation of the animal's threshold for the perception of and subsequent response to the stimulus.

Second, in most trials, particularly those at smaller (4 mm) apertures and at the lower irradiance values, the animals were monitored via the CCTV system. At the lower levels, no evidence of head withdrawal, blink rate increase, or other behavioral manifestations of avoidance were apparent. At the higher energy levels, in addition to the cessation of bar pressing behavior, the animals typically withdrew their heads, closed their eyes, or otherwise attempted to avoid the beam while they braced themselves for anticipated shock.

With the adjustment made for response baseline, the threshold for responses to the 16 mm beam was between 4 and 20 mw/cm^2, while the threshold for the 8 mm condition was found to fall between 25 and 50 mw/cm^2. In the former situation, the full beam was directed at the cornea, lids, and periocular areas of the rhesus monkey's face. The 8 mm aperture was positioned so that the center of the beam was centered on the cornea, while the outer edges of the beam were on the lids and lid margins. As can be seen from Figure 4, the sensitivity, as reflected by the mean SR's, was considerably lower for the 8 mm aperture condition.

When a 4 mm aperture was placed in the eyepiece and the beam directed to the center of the cornea, little suppression was noted either by evaluation of the animals' bar pressing behavior or by observation of their avoidance reactions. As a check on the relative sensitivity of this condition, the beam was directed to the hairless area directly below the lower lid and nasal to the eye. At both the 100 and 200 mw/cm^2 conditions, suppression occurred with no statistical difference between the mean SR's for the two values.

Responses occurring at the 4, 8, and 16 mm beam conditions appear to be directly related to both the size of the area stimulated and the tissues involved. While it was apparent that no sensitivity as measured by SR's was present to heat directed at the cornea in the 4 mm condition, the amount of corneal contribution to the 8 mm sensitivity data could be inferred by comparing the 200 mw/cm^2 cornea and lid data with the 200 mw/cm^2 data obtained from the face alone. No differences were seen in the responses under these two conditions. Thus in the present study, the cornea was not found to be sensitive to radiation from the CO_2 system under any of the area conditions. Lele and Weddell (9) presented a summary of their study in which near infrared radiation was used to stimulate the human corneas. Their study describes the verbal or perceptual problems encountered

by the subjects who described the stimulus variously as "itch," "tickle," and "pure warmth." These qualitative judgments were different from those evoked by stimulation of the skin with the same radiation. The disagreement between these past findings and those in the present study might be accounted for by evaluation of several parameters which differed between the two studies.

1. In the present study, rhesus monkeys were trained to detect radiation from the CO_2 laser emission. They were initially trained with the 8 mm aperture at 200 mw/cm^2. Because of repeated (over 1,000) exposures, stimulus generalization should have occurred if the sensation was the same at the cornea for the 4 mm aperture as for the cornea and lids with the larger beam diameters.

2. No avoidance behavior was seen in the rhesus monkey which would justify either the interpretation of heat sensation at the cornea or general irritation due to drying of the corneal surface.

3. A near infrared (1-3 μ) filtered stimulus was used with beam diameters of 2, 3, and 5 mm by Lele and Weddell at both 1 and 2 cal/second/cm^2 rates. These levels coincide to approximately 239 mw/cm^2 and 478 mw/cm^2. Since 35% of this energy enters the eye, it is conceivable that elements sensitive to heat in other structures such as the iris and ciliary body would be affected. In the present study no such confounding was present since more than 95% of the radiation was absorbed in the first 50 μ of the corneal epithelium.

The finding that rhesus monkeys can respond to irradiance as low as 4 mw/cm^2 for a 16 mm diameter beam again indicated the greater sensitivity of behavioral methods as opposed to tissue damage criteria data. The 100 mw/cm^2 safe viewing recommendations promulgated in TB Med 279 (4) appear to be correct. In no instance was any damage to the tissues of the cornea, lids, and face noted during or after more than 3,000 exposures at energy levels ranging from 1 to 200 mw/cm^2.

In a field situation in which an individual is exposed to CO_2 irradiation, the beam diameter will generally be large compared to those values used in the present study. Therefore, sensation of heat on the face or other parts of the body at low irradiance levels could serve as a warning to the individual that he had intercepted the beam.

SUMMARY AND CONCLUSIONS

Rhesus monkeys were trained to report the presence of heat produced by a CO_2 laser system. Four, 8, and 16 mm diameter beams were directed at the cornea, lids, and face of the animals at irradiances ranging from 1 to 200 mw/cm^2, and the results compared to those of other investigators who utilized human subjects. Several conclusions can be made based upon this study. (1) The cornea does not appear to be sensitive to heat produced by a CO_2 laser system at irradiances twice the recommended safety level. (2) No changes were observed in the corneas, lids, or facial tissues of the animals after several thousand 20-second exposures at these irradiances. (3) The threshold for sensitivity to CO_2 laser radiation with the 8 mm diameter beam was between 25 and 50 mw/cm^2, while for the 16 mm beam condition it was between 4 and 20 mw/cm^2. (4) No differences in the rhesus monkey responses were observed between the 8 mm beam directed at the cornea and lids and the same beam directed to a non-hairy area of the face, while responses to the 4 mm diameter beam were different when skin exposures were compared to corneal exposures.

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In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council.

REFERENCES

1. Byer, H.H., E. Carpino, and B. Stuck: Determination of the thresholds of CO_2 laser corneal damage to owl monkeys, rhesus monkeys, and Dutch belted rabbits. Frankford Arsenal Report M72-31, Feb. 1972, DDC AD-659 347.

2. Fine, B.S., S. Fine, L. Feigen, and D. MacKeen: Corneal injury threshold to carbon dioxide laser irradiation, Amer. J. Opth. 66:1-15, 1968.

3. Peabody, R.R., H.C. Zweng, H.W. Rose, N.A. Peppers, and A. Vassiliadis: Threshold damage from CO_2 lasers. Arch. Opth. 82:105-107, 1969.

4. Control of Hazards to Health from Laser Radiation. Department of the Army Technical Bulletin TB MED 279, Headquarters, Department of the Army, Washington, DC, 30 May 1975.

5. Harwerth, R.S., and H.G. Sperling: Prolonged color blindness induced by intense spectral lights in rhesus monkeys. Science 174, October 1971.

6. Brownell, A.S., and B. Stuck: Ocular and skin hazards of CO_2 laser radiation. Procedings of the Ninth Army Science Conference, OCRD, DA, Washington, DC, 1:123-137, 1974.

7. Zander, E., and G. Weddell: Observations on the innervation of the cornea. J. Anat. 85:68-99, 1951.

8. Von Frey, M.: Druckempfindung and Schmerz. Abh. sachs. Ges. Akad. Wiss. 23:175-266, 1896.

9. Lele, P.P., and G. Weddell: The relationship between neurohistology and corneal sensibility. Brain 79:119-154, 1956.

10. Lele, P.P., and G. Weddell: Sensory nerves of the cornea and cutaneous sensibility. Exp. Neur. 1:334-359, 1959.

11. Kenshalo, D.R.: Comparison of the sensitivity of the forehead, lip, conjunctiva and cornea. J. Appl. Physiol. 15:987-991, 1960.

12. Berkley, K.J., and S.S. Hughes: Identification of temperature increases and decreases: A difficult task for monkeys and humans. Experientia 28:798-799, 1972.

13. Smith, J.: Conditioned suppression as an animal psychophysical technique: in Stebbins, W.C., Ed., Animal Psychophysics, Appleton-Century-Crofts.

14. Laursen, A.M.: Thermal thresholds in man and monkeys. Exp. Neur. 34:25-32, 1972.

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